UNIVERSITY OF TECHNOLOGY SYDNEY

Gold nanoparticles to ameliorate obesity related glucose intolerance

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Abstract

Obesity is associated with a number of metabolic disorders such as insulin resistance, glucose intolerance, and dyslipidaemia. The main objective of this research work focused on potential therapeutic applications of "naked" gold nanoparticles (AuNPs) in the treatment of obesity-related metabolic diseases. In this thesis, 21 nm spherical citrate-coated, unmodified also known as "naked" AuNPs were studied and assessed using both *in vitro* cell and *in vivo* animal studies. We observed an increased rate of "naked" AuNPs cellular uptake non-selectively and non-specific accumulation in all cell types studied compared to surface modified AuNPs. Macrophages in adipose tissue played a crucial role contributing to the low-grade chronic inflammation present in obesity and pathogenesis of obesity-related metabolic disorders. Our *in vitro* studies also showed that AuNPs can act on the complex adipocyte-macrophage interaction altering the adipocytes lipid and fatty acid metabolic markers in a macrophage-dependent manner.

Based on this unique finding, we further investigated the long-term safety of AuNP treatment and their effect on adipose tissue macrophages using *in vivo* mice model with dietary obesity. In this study, 7 week old male C57Bl/6 mice were fed a high fat diet (HFD) and received daily intraperitoneal injection of AuNPs (0.785 μg or 7.85 μg/g/day) for 9 weeks. Our results showed that the AuNP-treated mice with dietary obesity showed significantly improved body morphometry measurement with significantly reduced blood lipid levels and prevented the development of glucose intolerance. We proposed that the alterations in the local pro-inflammatory cytokine environment due to modification of macrophage activity by AuNPs may be the key underlying mechanism for the weight loss observed in the HFD-fed mice. In addition, there was no indication of liver toxicity after 9 weeks of daily AuNPs exposure.

Following that, male C57Bl/6 mice (7 week old) feeding a 10 week HFD were used as an *in vivo* obese model with chronic obesity and metabolic disorder to study the effect of short-term AuNP treatment. At week 10, the obese mice were continued on a HFD and AuNP treatment (0.0785 μg, 0.785 μg, or 7.85 μg/g/day) administered daily via intraperitoneal injection for an additional 5 weeks. Our findings showed that the 5

weeks AuNP-treated obese mice had little to no impact on body morphometric measurements of mice with existing obesity. However, all obese mice treated with 5 weeks of AuNPs showed amelioration of glucose intolerance. Our results also showed altered the inflammatory cytokines, lipid, and glucose marker expression in the liver. Our findings suggest that obese mice treated with AuNPs could have a lower the risk of developing obesity-related complications such as glucose intolerance and liver steatosis.

This research work demonstrated the anti-obesity and anti-diabetic properties of AuNPs which has led to our proposal that AuNPs can serve as a novel therapeutic treatment strategy in the prevention of obesity and obesity-related metabolic disorders.

Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

(Jane Phui Mun Ng)

Date:

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Abbreviations

AC	adipocytes control
AF594	Alexa Fluor 594 cadevarine
AF594	Alexa Fluor 594 cadaverine
AH	adipocytes treated with high dose of AuNPs
AHH	adipocytes treated with extremely high dose of AuNPs
AL	adipocytes treated with low dose of AuNPs
ALT	alanine aminotransferase
ANOVA	analysis of variance
ARMΦC	adipocyte-macrophage co-culture control
ARMΦH	adipocyte-macrophage co-culture treated with high dose of AuNPs
ARMΦHH	adipocyte-macrophage co-culture treated with extremely high dose of AuNPs
ARMΦL	adipocyte-macrophage co-culture treated with low dose of AuNPs
AST	aspartate aminotransaminase
ATGL	adipose triglyceride lipase
ATM	adipose tissue macrophage
AUC	area under the curve
$AuCl_4$	chloroaurate
AuNP	gold nanoparticle
BAEC	bovine aortic endothelial
BMI	body mass index
BSA	bovine serum albumin

BSA-AuNP	BSA-conjugated AuNPs
CD	cluster of differentiation
cDNA	complementary DNA
Chow-C	chow-fed mice control
CLS	crown-like structure
CPT	carnitine palmitoyl transferace
DCF	2’,7’-dicholorofluorescein
DEPC	diethylpyrocarbonate
DL	detection limit
DMEM	dulbecco's modified eagle's medium
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxyucleotide
DPBS	dulbecco's PBS
EDTA	ethylenediaminetetraacetic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
F4/80	expressing ATMs
FASN	fatty acid synthase
FBS	fetal bovine serum
FOX	forkhead box
GLUT	glucose transporter
GM-CSF	granulocyte-macrophage colony-stimulating factor
H&E	harris haematoxylin and eosin

H_2-DCFDA	2',7'-dicholorofluorescein diacetate
$HAuCl_4$	tetrachlorouauric acid
HCAEC	human coronary artery endothelial cell
HFD	high fat diet
HFD-C	HFD-fed mice control
HFD-HAu	HFD-fed mice treated with high doses AuNPs
HFD-LAu	HFD-fed mice treated with low dose of AuNPs
HRP	horseradish peroxidase
HR-SEM	high resolution scanning electron microscopy
ICP-MS	inductively–coupled plasma mass spectroscopy
IFN	interferon
IKK	inhibitory kappa kinase
IL	interleukin
IP	intraperitoneal
IPGTT	intraperitonoteal glucose tolerance test
IκB	inhibitory kappaB
LPS	lipopolysaccharide
M1	pro-inflammatory macrophage
M2	anti-inflammtory macrophage
MES	2-(*N*-morpholino)ethanesulfonic acid
MΦ	macrophage
MPC	monolayer protected cluster
mRNA	messenger RNA

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCS	new calf serum
NEFA	non-esterified fatty acid
NF-κB	nuclear factor-kappa B
NHS	N-hydroxysuccinimide
OB-C	obese mice control
OB-EAu	obese mice treated with extremely low dose of AuNPs
OB-HAu	obese mice treated with high dose of AuNPs
OB-LAu	obese mice treated with low dose of AuNPs
P/S	penicillin and streptomycin
PBS	phosphate buffered saline
PC	pre-adipocyte or 3T3-L1 fibroblast control
PCR	polymerase chain reaction
PEG	polyethelyne glycol
PEPCK	phosphoenolpyruvate carboxykinase
pf-DMEM	phenol-red free Dulbecco's Modified Eagle Medium
PMA	phorbol 12-myristate 13-acetate
PPAR	peroxisome proliferator-activated receptor
RMΦ	RAW 264.7 macrophages
RMΦC	RAW 264.7 macrophages control
RMΦH	RAW 264.7 macrophages treated with high dose of AuNPs
RMΦHH	RAW 264.7 macrophages treated with extremely high dose of AuNPs

RMΦL	RAW 264.7 macrophages treated with low dose of AuNPs
RMΦLPSH	high dose LPS stimulated RAW 264.7 macrophage
RMΦLPSL	low dose LPS stimulated RAW 264.7 macrophages
RNA	ribonucleic acid
ROS	reaction oxygen speacies
RPMI	roswell park memorial institute
RT-PCR	real-time PCR
S.E.M	standard mean of error
SAA	serum amyloid A
SPR	surface plasmon resonance
SREBP	sterol regulatory element binding protein
TEM	transmission electron microscopy
TLR-4	toll-like receptor-4
TNF	tumor necrosis factor
UMΦ	U937 monocytes
UMΦC	PMA-differentiated U937 control
UMΦC_LPS	PMA-differentiated U937 with LPS stimulation control
UMΦH	PMA-differentiated U937 treated with high dose AuNP
UMΦH_LPS	PMA-differentiated U937 treated with high dose AuNPs and LPS
UMΦ_HH	PMA-differentiated U937 treated-with extremely high dose AuNPs
UMΦ_HH_LPS	PMA-differentiated U937 treated with extremely high dose AuNPs and LPS
UMΦL	PMA-differentiated U937 treated with low dose AuNPs